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SAN FRANCISCO, CALIFORNIA 94143

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Dear Mike:

As you know, I will be coming to the ICRF for a year next summer, and I am currently giving some thought to the sort of work I might do. Among the leading possibilities are several experiments outlined by the NIH guidelines. I am curious about your assessment of whether such things would be permitted by GMAC and whether they would be appropriate to do at ICRF. I am particularly interested in cloning the integration site at the 5' end of properly-oriented proviruses of avian sarcoma (ASV) and mouse mammary tumor virus (MMTV). In the case of ASV, we already have quite good evidence in a number of clones of transformed chicken cells that most if not all of the proviruses have recombined with the host genome at the appropriate point on the viral genome to permit synthesis of viral RNA. The ambiguous evidence we had about tandom integration has been resolved against the tandem model by the use of additional enzymes and the discovery of a fourth Eco RI site very near the 5' end of the genome (there is also one, you may recall very near the 3' end, making the initial mapping difficult). In some clones we have produced an Eco RI fragment of 0.8×10^6, containing about 0.2×10^6 daltons of information from the 5' end of the viral genome and 0.6×10^6 of cell DNA. This sort of fragment would seem ideal for cloning, since it is well removed from the "dangerous" part of the viral genome (src), has a limited amount of viral information (most of which is either non-coding or coding for the p19 product of the gag gene), and is likely to contain important information in the cellular sequence (e.g., part of any recognition signal for integration, possibly a cellular promoter for viral RNA synthesis, etc.). The viral part of the "integration fragment" can be further trimmed, since the 5' sequence is known and contains several restriction sites. We are slightly less far along with MMTV, but we have an extensive map of the unintegrated DNA and know a few things about integration (it is not tandem, there are at least several possible sites). However, the MMTV site(s) is (are) of particular interest in view of the strong regulation of viral gene expression by glucocorticoids; it is possible that the provirus integrates adjacent to a regulatory site in the host DNA. Again, we hope within the next few months to have the 5' viral sequence and to know more about "integration fragments", so it should be possible to identify those fragments most worth cloning.

The MMTV genome is less well mapped genetically, of course; but, hopefully, the viral portion of any fragment could be trimmed to 50 nucleotides or less. It has always seemed simpler and more efficient to me to clone such fragments in E. coli, provided permission were obtainable, but I would like to have your view of the advisability of cloning this sort of DNA in polyoma. In particular, with reference to MMTV, do you think the argument that the viral genomes are likely to interact in nature would carry any weight with GMAC?

As I mentioned to you when I was in London, Phil Coffino, Herb Boyer and I have been discussing for some time the possibility of transfecting a TK mouse cell with recombinant DNA made from polyoma tsA DNA (also lacking a portion of the late region) and from a restriction fragment of pseudorabies virus DNA bearing the TK gene. The idea would be to grow the TK-transformed cell at the non-permissive temperature (to prevent replication of the hybrid DNA), then to make various kinds of TK mutants with various agents and obtain the relevant of the DNA from each mutant by shifting to temperature permissive for replication. Unfortunately, this experiment is still initially P4 in this country---we have even had a specific appeal denied---but I wonder whether you think it would pass GMAC and whether you think it is a feasible (or sensible) approach to studying eukaryotic mutants. Although this project is primarily Phil's, he would be delighted if I could accomplish the initial stages abroad, to allow the requirements to be reduced to P3 after we have defined what we've cloned.

Thanks again for taking the time to give me a tour during my April visit; our conversation strongly influenced my desire to come to the ICRF next year. I look forward to hearing your views on the above issues; and I would also appreciate having some sense of the timing required to have GMAC consider any request for permission to do recombinant work starting the summer of 1978.

Best regards to you and others on the fifth floor,

Harold E. Varmus, M.D.
Associate Professor
Department of Microbiology

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